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BLACKLEG TOXIN

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The production of toxin by B. chauveaui has long been a mooted question. Roux,[1] Duenschmann,[2] and later Leclainche and Vallee,[3] reported the formation of toxic substances in cultures of blackleg. Leclainche and Vallee[3] showed that filtrates of 5-day old cultures of blackleg grown on Martin's bouillon were able to kill guinea-pigs promptly in doses of 5 c c, injected intraperitoneally. They did not succeed in immunizing guinea-pigs with this toxin, because doses that were not immediately fatal produced severe emaciation and death in 1 or 2 weeks. Leclainche and Vallee concluded from their experiments that the serum from horses immunized with cultures grown on Martin's bouillon, although strongly anti-infectious, is not antitoxic, because guinea-pigs made passively immune against infection by this serum regularly succumbed to the toxic effects of a dose of blackleg culture, witht the same symptoms as those receiving only toxin. Later, Foth[4] showed that cultures of blackleg produced soluble immunizing substances. Leclainche and Vallee, and also Foth, carefully established the identity of their cultures, especially distinguishing between B. chauveaui and "vibrion septique" (bacillus of malignant edema), and therefore their observations warrant careful consideration.

Recently Nitta,[5] Eichhorn,[6] and Kelser[7] have made claims in regard to the production of toxin in cultures of blackleg, the latter holding that the immunizing properties of the filtrate are in direct relation to its toxicity, and that nontoxic filtrates have no value as immunizing agents. As no data are furnished by these authors in regard to the purity and identity of the strains used, their publications cannot be considered authoritative. The experiences of Schattenfroh and Grass-

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[1] Ann. de l'Instit. Pasteur, 1888, 2, p. 49.

[2] Ibid., 1894, 8, p. 40.

[3] Ibid., 1900, 4, p. 202 and p. 590.

[4] Ztschr. f. Infectionskrankh. der Hausthiere, 1911, 10, p. 1; ibid., 909, 201.

[5] Jour. Am. Vet. Med. Assn., 53, n. s. 1918, 6, p. 466.

[6] Ibid., 52, n. s., 1918, 5, p. 653.

[7] Jour. Agri. Research, 1918, 14, p. 253.

berger with cultures of blackleg and other somewhat similar anaerobes emphasize the extreme necessity of careful purity and identity tests. Schattenfroh and Grassberger alone of the authors mentioned demonstrated that the poisonous substances occurring in their cultures were true toxins, capable of producing antitoxins, and not products of metabolism, and were justified in using the term toxin. However, von Hibler[8] and others hold that in all probability Schattenfroh and Grassberger were confusing Fraenkel's bacillus with B. chauveaui.

PROPERTIES OF B. CHAUVEAUI

Without attempting to go minutely into the differential diagnosis of blackleg, it would seem worth while to briefly recount some of the most important properties of B. chauveaui, especially because of some of the statements appearing in recent literature. Thus, one author[9] finds that the filtrates prepared by a colleague were strongly alkaline. Eichhorn[6] records that the meat added to his blackleg culture was digested. The filtrates made by certain laboratories have exceeding foul odors. According to von Hibler,[10] blackleg produces an acid reaction, does not peptonize sterile meat in cultures, and does not produce a putrid odor. Th. Smith,[11] von Hibler,[10] Leclainche and Vallee,[3] and Foth,[4] agree in every essential point as to the morphologic and cultural characteristics of B. chauveaui. Of the various reactions mentioned by these authors the following have proved especially useful in checking the purity and identity of cultures previous to their use in experimental work, and the strain of blackleg used in our experiments possessed these characteristics.

In peptone liver broth with cooked brain tissue a vigorous evolution of gas occurs within 24 hours, and the medium becomes moderately cloudy. In a few days evolution of gas ceases and the liquid part of the medium becomes as clear as before inoculation. There is no blackening of the medium and no formation of putrid odor even in cultures 2 weeks old or more. The cultures remain permanently acid. If the medium is highly alkaline at the start the organisms may not be able to continue growth until it is acid, but the alkalinity will be at least neutralized.

Emulsion of a 24-hour culture in dose of 0.1 c c will usually kill guineapigs which show at the point of inoculation a large area of hemorrhagic edema without putrid odor or evidence of peptonization or digestion of tissue. Other related pathogenic anaerobes produce lesions very similar and differentiation on this ground is not attempted. Smears made from the diaphragmatic surface of the liver after death with blackleg show slender rods singly or in pairs, rarely in fours. This furnishes a valuable means of differentiating from B. edematis-maligni which produces long chains on the serous membranes although its form in the muscle tissue may very closely resemble blackleg.

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[8] Article by Felix v. Werdt in Handbuch der Pathogenen Microorganismen by Kolle and Wassermann, 1912, 4, p. 902.

[9] Berg, William N.: Jour. Agri. Research, 1918, 14, p. 264.

[10] Handbuch der Pathogenen Microorganismen by Kolle and Wassermann, 1912, 4, p. 800.

[11] Ztschr. f. Infektionskrankh. der Haustiere, 1905, 1.

As a final test of the identity of a strain showing these characteristics, a test is made with a serum made by another laboratory. In our work 3 guinea-pigs each receive 0.6 c c of the blackleg serum made by Institut de Serotherapie de Toulouse, Professors Leclainche and Vallee. Twenty-four hours later these guinea-pigs and 3 check guinea-pigs receive 0.5 c c each of an emulsion of the culture to be tested. If the guinea-pigs receiving the serum live and the checks die the identity of the blackleg strain is considered sufficiently established.

MEASUREMENT OF TOXICITY ON GUINEA-PIGS AND IMMUNIZATION OF CALVES

It was the plan of the present experiment to test the toxic powers on guinea-pigs of filtrates of blackleg cultures made in this and other laboratories, and to test their immunizing power on calves, in order to ascertain if any relation existed between toxicity on guinea-pigs and immunizing power for calves.

TABLE 1

MEASUREMENT OF TOXICITY ON GUINEA-PIGS OF BLACKLEG FILTRATES FROM FOUR DIFFERENT LABORATORIES

Guinea-Pig	Injection	Blackleg Filtrate C C	Result
		Group 1—Filtrate A	
1	Subcutem	10.0	Slight swelling
2	Subcutem	10.0	Marked swelling
3	Subcutem	10.0	Slight swelling
4	Subcutem	10.0	Slight swelling
		Group 2—Filtrate B	
1	Intraperitoneal	5.0	Slight swelling
2	Intraperitoneal	5.0	Slight swelling
		Group 3—Filtrate C	
1	Intraperitoneal	5.0	Marked swelling
2	Intraperitoneal	5.0	Marked swelling
		Group 4—Filtrate D	
1	Subcutem	10.0	Severe swelling, sick
2	Subcutem	10.0	Marked swelling
3	Subcutem	10.0	Marked swelling
4	Subcutem	10.0	Marked swelling
5	Intraperitoneal	10.5	Marked swelling
6	Intraperitoneal	10.0	Slight swelling
7	Muscles of leg	5.0	Slight swelling
8	Muscles ofleg	5.0	Slight swelling

TABLE 2

IMMUNIZATION OF CALVES AND TEST FOR IMMUNITY

The dose of filtrate was 5 c c and of virus 10 c c.

Group 1—Filtrate A	
1	Lived
2	Lived
3	Lived
Group 2—Filtrate B	
1	Lived
2	Died
3	Lived
4	Died
Group 3—Filtrate C	
1	Lived
2	Lived
3	Lived
4	Lived
Group 4—Filtrate D	
1	Lived
2	Lived
3	Lived

Ten nontreated calves receiving same dose of virus all died.

The filtrates tested were usually given subcutaneously in the region of the pectoral muscles and the adjacent axilla which allows 5 or 10 c c to be injected and absorbed with ease. As a check on the method of inoculation some animals were injected in the muscles of the leg, and some intraperitoneally. Table 1 gives a summary of the effect on guinea-pigs of blackleg filtrates from 4 different laboratories, and it is seen that all of the filtrates were nontoxic, none of them being able to produce death or serious symptoms in guinea-pigs when given in doses of 5 or 10 c c.

All of the calves in this experiment received 5 c c of blackleg filtrate each and after at least 2 weeks were tested for immunity by inoculation with a culture of blackleg carefully tested for purity as already detailed. The result of the test with calves is given in Table 2, from which it is seen that of 14 calves treated with filtrate only two were later susceptible to virus. Ten controls receiving virus alone, all died of blackleg. It will be noted that the filtrates from the 4 different laboratories possessed marked immunizing properties. It is not within the scope of this paper to compare the efficiency of the various filtrates, and no inquiry was made as to their method of production, so on that point no statement can be made. They were all believed by their makers to be efficient immunizing agents against blackleg, and this contention was in the main confirmed, in spite of the fact that they in no instance possessed any marked toxicity.

SUMMARY

Blackleg filtrates made by four different laboratories were nontoxic to guinea-pigs, and possessed marked immunizing properties in calves. The experiments indicate that the immunizing power of blackleg filtrates is not dependent on toxicity.